Applicants: Stemmler et al.

Serial No.: 09/492,214

Filing Date: January 27, 2000 Amendment and Reply to Nonfinal Office Action

June 15, 2004 Page 3 of 17

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the

application.

**Listing of Claims:** 

1. (canceled)

2. (previously presented) The method according to Claim 44 in which the method is an

affinity assay.

3. (previously presented) The method according to Claim 42 in which the analyte

comprises a nucleic acid.

4. (previously presented) The method according to Claim 44 in which the method is an

immuno-affinity assay.

5. (previously presented) The method according to Claim 42 in which the analyte

determination occurs in a volume of less than 1 µl.

6. (previously presented) The method according to Claim 44 in which the method is a

competitive assay.

7. (previously presented) The method according to Claim 44 in which the method is a

sandwich assay.

8. (canceled)

Applicants: Stemmler et al.

Serial No.: 09/492,214

Filing Date: January 27, 2000 Amendment and Reply to Nonfinal Office Action

June 15, 2004 Page 4 of 17

9. (previously presented) The method according to Claim 42 in which the measurement

signal is generated by irradiation excitement of the bound labeled reagent.

10. (previously presented) The method according to Claim 42 in which the labeled

reagent is a fluorescent labeled reagent.

11. (previously presented) The method according to Claim 42 in which the sample is in

a liquid phase.

12. (previously presented) The method according to Claim 42 in which the solid phase

is formed on a wall of a well in a sample carrier.

13. (previously presented) The method according to Claim 12 in which the carrier is a

micro-titre or nano-titre plate.

14. (previously presented) The method according to Claim 12 in which the well has a

quadratic, cylindrical, truncated pyramid or truncated cone shape.

15. (previously presented) The method according to Claim 12 in which the well has an

aperture area and a floor area, the aperture area being smaller than the floor area.

16. (previously presented) The method according to Claim 15 in which the well has a

truncated pyramid or truncated cone shape.

17. - 18. (canceled)

19. (previously presented) The method according to Claim 42 in which the measurement

Applicants: Stemmler *et al.* Serial No.: 09/492,214

Filing Date: January 27, 2000

Amendment and Reply to Nonfinal Office Action

June 15, 2004 Page 5 of 17

signal is obtained by spatially staggered measurement.

20. (canceled)

21. (previously presented) The method according to Claim 42 in which a light beam is used to excite the sample, said light beam having a diameter of less than 40 µm.

22. (canceled)

23. (previously presented) The method according to Claim 21 in which a laser provides the light beam.

24. - 32. (canceled)

33. (previously presented) The method according to Claim 5 in which the volume is in the range of 50 to 100 nl.

34. (previously presented) The method according to Claim 13 in which the sample carrier is a nano-titre plate.

- 35. (previously presented) The method according to Claim 42 in which the quenching substance is a metal, dye or fluorescence-quenching substance.
- 36. (previously presented) The method according to Claim 23 in which the light beam has a diameter of about 20  $\mu$ m.

37. - 41. (canceled)

Applicants: Stemmler *et al.* Serial No.: 09/492,214

Filing Date: January 27, 2000

Amendment and Reply to Nonfinal Office Action

June 15, 2004 Page 6 of 17

42. (currently amended) A method for quantitative or qualitative determination of an analyte comprising:

- (a) incubating a sample containing the analyte with labeled reagent and a solid phase coated with a quenching substance so as to allow at least some of the labeled reagent to bind to the analyte, wherein the quenching substance is capable of suppressing suppresses at least some of the signal from unbound labeled reagent;
- (b) exciting the sample so as to generate signal from the bound labeled reagent; and
- (c) measuring the signal generated from the bound labeled reagent, thereby quantitatively or qualitatively determining the analyte, wherein the determination of the analyte occurs is performed or effected without physically separating unbound and bound labeled reagent.
- 43. (previously presented) The method according to Claim 42, wherein the quenching substance is gold, silver or graphite.
- 44. (previously presented) The method according to Claim 42, wherein the labeled reagent is selected from the group consisting of antigen, antibody, nucleic acid, ligand or receptor.
- 45. (previously presented) The method according to Claim 42, wherein the labeled reagent is immobilized on the solid phase.
- 46. (withdrawn) A method for quantitative or qualitative determination of an analyte comprising:
- (a) incubating a sample containing the analyte with labeled reagent and a solid phase coated with a metal quenching substance so as to allow at least some of the labeled reagent to bind to the analyte, wherein the metal quenching substance is capable of

Applicants: Stemmler *et al.* Serial No.: 09/492,214

Filing Date: January 27, 2000

Amendment and Reply to Nonfinal Office Action

June 15, 2004 Page 7 of 17

suppressing signal from unbound labeled reagent;

- (b) exciting the sample so as to generate signal from the bound labeled reagent; and
- (c) measuring the signal generated from the bound labeled reagent, thereby quantitative or qualitative determining the analyte, wherein the determination of the analyte occurs without physically separating unbound and bound labeled reagent.
- 47. (currently amended) A method for quantitative or qualitative determination of a labeled analyte comprising:
- (a) incubating the <u>a</u> sample containing labeled analyte with a reagent and a solid phase coated with a quenching substance so as to allow at least some of the labeled analyte to bind to the reagent, wherein the quenching substance is capable of suppressing suppresses at least some signal from unbound labeled analyte;
- (b) exciting the sample so as to generate signal from the bound labeled analyte; and
- (c) measuring the signal generated from the bound labeled analyte, thereby quantitative or qualitative quantitatively or qualitatively determining the labeled analyte, wherein the determination of the labeled analyte occurs is performed or effected without physically separating unbound and bound labeled analyte.
- 48. (new) A method for quantitative or qualitative determination of an analyte, comprising:
- (a) incubating a sample containing an analyte with a labeled reagent and a solid phase comprising a bonding partner for the analyte so as to allow at least some of the analyte to bind to the bonding partner and at least some of the labeled reagent to bind to the analyte, wherein the labeled reagent is in a liquid phase and the solid phase is coated with a quenching substance that suppresses at least some signal from bound labeled reagent;

Applicants: Stemmler *et al.* Serial No.: 09/492,214

Filing Date: January 27, 2000

Amendment and Reply to Nonfinal Office Action

June 15, 2004 Page 8 of 17

(b) exciting the sample so as to generate signal from the labeled reagent; and

(c) measuring signal from labeled reagent in the liquid phase, thereby quantitatively or qualitatively determining the analyte, wherein the determination of the analyte is performed or effected without physically separating unbound and bound

labeled reagent.

49. (new) A method according to claim 48, wherein the analyte comprises an antigen

and the bonding partner and labeled reagent comprises an antibody.

50. (new) A method according to claim 48, wherein the label is a fluorescent label.

51. (new) A method according to claim 48, wherein the quenching substance is a metal,

dye or fluorescence-quenching substance.

52. (new) A method according to claim 48, wherein the quenching substance is gold,

silver, or graphite.

53. (new) A method for quantitative or qualitative determination of an analyte,

comprising:

(a) incubating a sample containing an analyte with a labeled bonding partner and

a solid phase comprising a second bonding partner so as to allow at least some of the

analyte and at least some of the labeled bonding partner to bind to the second binding

partner, wherein the labeled bonding partner is in a liquid phase and the solid phase is

coated with a quenching substance that suppresses at least some signal from bound

labeled bonding partner;

(b) exciting the sample so as to generate a signal from the labeled bonding

partner; and

(c) measuring the signal from the labeled bonding partner in the liquid phase,

Applicants: Stemmler et al.

Serial No.: 09/492,214

Filing Date: January 27, 2000

Amendment and Reply to Nonfinal Office Action

June 15, 2004 Page 9 of 17

thereby quantitatively or qualitatively determining the analyte, wherein the determination

of the analyte is performed or effected without physically separating unbound and bound

labeled bonding partner.

54. (new) A method according to claim 53, wherein the analyte comprises an antigen

and the labeled and second bonding partners comprise an antibody.

55. (new) A method according to claim 53, wherein the label is a fluorescent label.

56. (new) A method according to claim 53, wherein the quenching substance is a metal,

dye or fluorescence-quenching substance.

57. (new) A method according to claim 53, wherein the quenching substance is gold,

silver, or graphite.

58. (new) A method for quantitative or qualitative determination of an analyte,

comprising:

(a) incubating a sample containing an analyte and a labeled reagent with a solid

phase comprising a bonding partner so as to allow at least some of the analyte and at least

some of the labeled reagent to bind to the binding partner, wherein the labeled reagent is

in a liquid phase and the solid phase is coated with a quenching substance that suppresses

at least some of the signal from the labeled reagent;

(b) exciting the sample so as to generate a signal from the labeled reagent; and

(c) measuring the signal from the labeled reagent in the liquid phase, thereby

quantitatively or qualitatively determining the analyte, wherein the determination of the

analyte is performed or effected without physically separating unbound and bound

labeled reagent.

Applicants: Stemmler *et al.* Serial No.: 09/492,214 Filing Date: January 27, 2000

Amendment and Reply to Nonfinal Office Action

June 15, 2004 Page 10 of 17

- 59. (new) A method for quantitative or qualitative determination of an analyte, comprising:
- (a) incubating a sample containing an analyte with a labeled reagent, a first bonding partner for the analyte and a solid phase comprising a second bonding partner so as to allow at least some of the analyte to bind to the first and second binding partners and at least some of the labeled reagent to bind to the first bonding partner, wherein the labeled reagent is in a liquid phase and the solid phase is coated with a quenching substance that suppresses at least some of the signal from bound labeled reagent;
  - (b) exciting the sample so as to generate a signal from the labeled reagent; and
- (c) measuring the signal from the labeled reagent in the liquid phase, thereby quantitatively or qualitatively determining the analyte, wherein the determination of the analyte is performed or effected without physically separating unbound and bound labeled reagent.